

## SUPPLEMENTAL MATERIAL

### Selective Pressure of Antibiotic Pollution on Bacteria of Importance to Public Health

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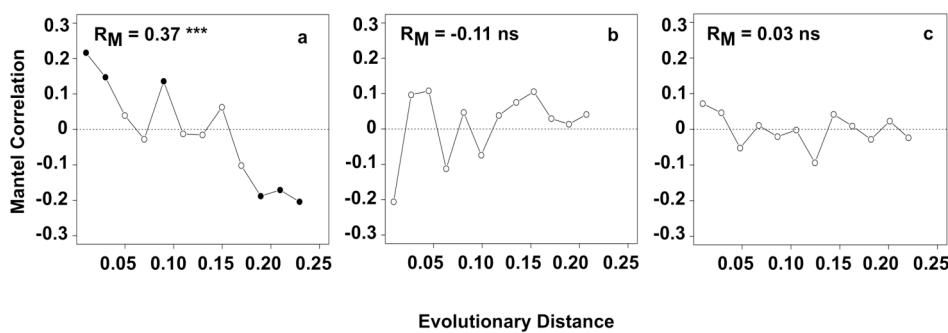
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## Supplemental Material, Section S1

### Correlation structure of MIC datasets

An implicit assumption in the use of SSDs is that taxa used to derive them represent independent observations drawn from a random distribution (Suter et al. 2002). To minimize the lack of independence between observations, several methods and researchers recommend combining the endpoints from co-generic species (see Suter et al. 2002). Mantel tests (Mantel 1967; Legendre and Legendre 1998) were used to explore the correlation between evolutionary distances and pairwise differences in median MICs between taxa for each antibiotic dataset. Figure 1 shows Mantel correlograms for each antibiotic, along with the Mantel correlation coefficient for the entire dataset in the upper left hand side of each graph (i.e.,  $R_M$ ). Grouping data from co-generic species to minimize the lack of independence before SSD derivation seemed advisable for the ciprofloxacin dataset, whereas there did not seem to be a statistical ground for doing so in the erythromycin and tetracycline datasets. In this study we decided to group co-generic species in all datasets in order to apply a consistent methodology in the derivation of SSDs for all three antibiotics that would facilitate the subsequent interpretation and discussion of results.



**Supplemental Material, Figure S1.** Mantel correlograms describing the correlation between  $\text{MIC}_{50}$  and evolutionary distance for ciprofloxacin (a), erythromycin (b) and tetracycline (c). Solid dots represent a significant correlation at the corresponding evolutionary distance ( $\alpha = 0.05$ ). Significance tests are based on 5000 permutations.

## Supplemental Material, Table S1

Taxa represented in EUCAST MIC datasets. Cross (+) and dash (-) symbols indicate presence or absence of each entry in the given EUCAST MIC antibiotic dataset, respectively. The All-species Living Tree Project names of the subset of species which were represented in the LTP 16S rRNA database are given in the ‘LTP Name’ column. MIC data from co – generic species in each dataset were pooled to derive SSDs.

Species	Ciprofloxacin	Erythromycin	Tetracycline	LTP Name
<i>Acinetobacter anitratus</i>	+	-	-	
<i>Acinetobacter baumanii</i>	+	-	+	AcnBau29
<i>Acinetobacter lwoffii</i>	+	-	-	AcnLwo34
<i>Acinetobacter calcoaceticus</i>	+	-	-	AcnCal77
<i>Acinetobacter spp</i>	+	-	+	
<i>Alcaligenes xylosoxidans</i>	+	-	-	
<i>Bacteroides fragilis</i>	+	-	-	BcdFra36
<i>Bacteroides fragilis group</i>	+	+	-	
<i>Bacteroides ovatus</i>	-	-	-	BcdOvat8
<i>Bacteroides thetaiotaomicron</i>	-	-	-	BcdThe32
<i>Bacteroides vulgatus</i>	-	-	-	BcdVulg9
<i>Burkholderia cepacia</i>	+	-	-	BurCe154
<i>Bifidobacterium longum</i>	-	+	+	BifLon13
<i>Bifidobacterium pseudolongum</i>	-	-	+	BifPseu8
<i>Bifidobacterium thermophilum</i>	-	-	+	
<i>Campylobacter coli</i>	+	+	+	CampCol5
<i>Campylobacter jejuni</i>	+	+	+	CamJejun
<i>Chryseobacterium meningosepticum</i>	+	-	-	
<i>Chryseobacterium spp</i>	+	-	-	
<i>Citrobacter braakii</i>	-	-	+	CitBraak
<i>Citrobacter freundii</i>	-	-	+	CitFre32
<i>Citrobacter koseri</i>	-	-	+	
<i>Citrobacter spp</i>	+	-	+	
<i>Clostridium difficile</i>	-	+	+	CloDiff5
<i>Clostridium perfringens</i>	-	+	-	CloPe172
<i>Enterobacter aerogenes</i>	+	-	+	EntAero9
<i>Enterobacter agglomerans</i>	+	-	+	
<i>Enterobacter cloacae</i>	+	-	+	EntClo58
<i>Enterobacter dissolvens</i>	+	-	+	EntClo52
<i>Enterobacter spp</i>	+	-	+	
<i>Enterococcus avium</i>	-	-	-	EnrAviu7
<i>Enterococcus casseliflavus</i>	-	-	-	EnrCas20
<i>Enterococcus faecalis</i>	+	+	+	EnrFa149
<i>Enterococcus faecium</i>	+	+	+	EnrFa232
<i>Enterococcus gallinarum</i>	-	-	-	EnrGall8

Supplemental Material, Table S1 (continued)

Species	Ciprofloxacin	Erythromycin	Tetracycline	LTP Name
<i>Enterococcus hirae</i>	-	+	+	EnrHir11
<i>Escherichia coli</i>	+	-	+	EschCo52
<i>Haemophilus influenzae</i>	+	+	+	HaeIn157
<i>Haemophilus parainfluenzae</i>	+	+	+	HaePara4
<i>Hafnia alvei</i>	+	-	+	HafAlv15
<i>Helicobacter pylori</i>	+	-	+	HelPyl55
<i>Klebsiella oxytoca</i>	+	-	+	KleOxy19
<i>Klebsiella pneumoniae</i>	+	-	+	KlePn101
<i>Klebsiella spp</i>	+	-	+	
<i>Kluyvera spp</i>	+	-	+	
<i>Lactobacillus lactis</i>	-	+	+	
<i>Legionella pneumophila</i>	+	+	-	LgnPne10
<i>Listeria monocytogenes</i>	+	-	+	LstMon13
<i>Manhemia haemolytica</i>	-	-	+	ManHaem8
<i>Moraxella catarrhalis</i>	+	+	+	MorCatar
<i>Morganella morganii</i>	+	-	+	MrgMor25
<i>Mycobacterium tuberculosis</i>	+	-	-	MycTube9
<i>Neisseria gonorrhoeae</i>	+	+	+	NeiGono5
<i>Neisseria meningitidis</i>	+	-	+	
<i>Pasteurella multocida</i>	+	+	+	PsuMult6
<i>Peptostreptococcus spp</i>	-	+	-	
<i>Propionibacterium acnes</i>	-	-	+	ProAcn42
<i>Proteus mirabilis</i>	+	-	+	PtsMir19
<i>Proteus vulgaris</i>	+	-	+	PtsVulg9
<i>Proteus spp</i>	-	-	+	
<i>Providencia rettgeri</i>	-	-	-	PrvRett3
<i>Providencia stuartii</i>	+	-	-	PrvStua5
<i>Providencia spp</i>	+	-	-	
<i>Pseudomonas aeruginosa</i>	+	-	+	PseAe290
<i>Pseudomonas fluorescens</i>	+	-	-	PseFl192
<i>Raoultella spp</i>	+	-	+	
<i>Raoultella spp+</i>	-	-	+	
<i>Salmonella enteritidis</i>	-	-	-	
<i>Salmonella paratyphi</i>	-	-	-	
<i>Salmonella typhi</i>	-	-	-	
<i>Salmonella typhimurium</i>	-	-	-	
<i>Salmonella spp</i>	+	-	+	
<i>Serratia liquefaciens</i>	+	-	+	SerLiqu3
<i>Serratia marcescens</i>	+	-	+	SerMa116
<i>Serratia spp</i>	+	-	+	
<i>Shigella sonnei</i>	-	-	-	StaAur61
<i>Staphylococcus aureus</i>	+	+	+	
<i>Staphylococcus aureus MRSA</i>	+	+	+	
<i>Staphylococcus aureus MSSA</i>	+	+	+	
<i>Staphylococcus auricularis</i>	+	-	-	StaAuri2
<i>Staphylococcus capitis</i>	+	+	-	StaCap14

Supplemental Material, Table S1 (continued)

Species	Ciprofloxacin	Erythromycin	Tetracycline	LTP Name
<i>Staphylococcus coagulase negative</i>	+	+	+	
<i>Staphylococcus coagulase negative MRSE</i>	+	+	+	
<i>Staphylococcus cohnii</i>	+	-	-	StaCohn9
<i>Staphylococcus epidermidis</i>	+	+	+	StaEpi72
<i>Staphylococcus epidermidis MSSE</i>	+	+	+	
<i>Staphylococcus haemolyticus</i>	+	+	+	StaHae18
<i>Staphylococcus hominis</i>	+	+	-	StaHom26
<i>Staphylococcus hyicus</i>	+	+	-	StaHyicu
<i>Staphylococcus intermedius</i>	+	+	-	StaInte4
<i>Staphylococcus lugdunensis</i>	+	-	-	StaLugd4
<i>Staphylococcus saprophyticus</i>	+	+	+	StaSap31
<i>Staphylococcus sciuri</i>	+	-	-	StaSci15
<i>Staphylococcus simulans</i>	+	-	-	StaSimul
<i>Staphylococcus warneri</i>	+	+	-	StaWarne
<i>Staphylococcus xylosis</i>	+	-	-	StaXyl10
<i>Stenotrophomonas maltophilia</i>	+	-	+	SteMa216
<i>Streptococcus acidominimus</i>	+	-	-	
<i>Streptococcus agalactiae</i>	+	+	+	StpAga25
<i>Staphylococcus anginosus</i>	+	+	+	StpAng35
<i>Streptococcus bovis</i>	+	-	-	
<i>Streptococcus constellatus</i>	+	-	-	StpCon17
<i>Streptococcus equinus</i>	+	-	-	StpEqui4
<i>Streptococcus gordonii</i>	+	-	-	StpGor11
<i>Streptococcus group C</i>	+	-	-	
<i>Streptococcus group G</i>	+	+	+	
<i>Streptococcus intermedius</i>	+	-	-	StpInte8
<i>Streptococcus milleri</i>	+	+	-	
<i>Streptococcus mitis</i>	+	-	-	StpMit32
<i>Streptococcus oralis</i>	+	+	+	StpOra18
<i>Streptococcus parasanguis</i>	+	-	-	StpPar15
<i>Streptococcus pneumoniae</i>	+	+	+	StpPne46
<i>Streptococcus pyogenes</i>	+	+	+	StpPyo39
<i>Streptococcus salivarius</i>	+	-	-	StpSali6
<i>Streptococcus sanguinis</i>	+	-	-	StpSan11
<i>Streptococcus thermophilus</i>	-	+	+	
<i>Streptococcus uberis</i>	+	-	-	StpUber5
<i>Streptococcus viridans</i>	+	+	+	
<i>Yersinia spp</i>	+	-	+	
<b>Total</b>	<b>91</b>	<b>40</b>	<b>67</b>	<b>79</b>
<b>16S rRNA sequences</b>	<b>63 (70%)</b>	<b>28 (70%)</b>	<b>43 (64%)</b>	

## Supplemental Material, Section S2

### Evidence for growth of selected genera in the environment

There is evidence to suggest that several bacteria of importance to public health may, under certain conditions, grow in different environments. Hendricks (1972), for example, showed that some *Enterobacteriaceae* could grow in water collected downstream of a municipal sewage facility at temperatures as low as 5°C. Gibbs et al. (1997) reported years later the re-growth of faecal coliforms and *Salmonella* in biosolids and soil amended with biosolids. The populations of *E. coli* in manured soils can be very dynamic (Topp et al. 2003) and Inglis et al. (2010) recently showed that *Campylobacter* can persist for long periods of time in compost, which may suggest cryptic growth. Enterococci have been shown to grow in municipal oxidation ponds (Moriarty et al. 2008), and the facultative intracellular pathogen *Listeria monocytogenes* has been shown to be widespread in certain catchments (Lyautey et al. 2007) and able to grow in soil suspensions characteristic of certain organic fractions (Sidorenko et al. 2006). Staphylococci, including *Staphylococcus aureus*, have been isolated from marine water samples (Gunn and Colwell 1983), and methicillin – resistant *Staphylococcus aureus* was recently isolated from marine water and intertidal sand from beaches on the west coast of the USA (Soge et al. 2009). *S. aureus* has also been shown to be capable of growth in sterile soil (Liang et al. 1982), suggesting that it might be possible for it to grow in this environment under conditions of low competition. Ayyadurai et al. (2008) recently demonstrated that *Yersinia pestis* remained viable and fully virulent in humid sand for 40 weeks. *Clostridium difficile* is widely distributed in the environment (Al Saif and Brazier 1996), and it is not unreasonable to speculate that there may be niches that could

support its sporulation. Collectively, these studies highlight there is a potential for growth in bacteria of clinical relevance in different environments and under varying biological and physicochemical conditions, even if only during a short temporal window. In the presence of antibiotics, wild-type populations may be inhibited to various extents, increasing the prevalence of resistance. Full references upon which we based our decision to include genera for SSD derivation are given in Table S2.

### **Supplemental Material, Table S2**

Bacterial genera represented in the SSDs. References provide either direct evidence of growth in the environment or evidence that suggests that under certain conditions it is possible for growth to occur.

	Genus	Reference
1	<i>Acinetobacter</i>	Madigan et al. 2009
2	<i>Alcaligenes</i>	Agerso et al. 2005; Madigan et al. 2009
3	<i>Burkholderia</i>	Madigan et al. 2009
4	<i>Campylobacter</i>	Brandl et al. 2004; Inglis et al. 2010
5	<i>Chryseobacterium</i>	Vandamme et al. 1994
6	<i>Citrobacter</i>	Madigan et al. 2009; Kitts et al. 1994
7	<i>Clostridium</i>	Al Saif and Brazier 1996; Madigan et al. 2009
8	<i>Enterobacter</i>	Hendricks 1972; Rattray et al. 1995; Hernandez et al. 1998
9	<i>Enterococcus</i>	Mundt 1961/1963; Desmarais 2002; Moriarty et al. 2008
10	<i>Escherichia</i>	Hendricks 1972; Gibbs et al. 1997; Topp et al. 2003; Zaleski et al. 2005
11	<i>Hafnia</i>	Jand 2006
12	<i>Klebsiella</i>	Liang et al. 1982; Zadoks et al. 2011 (and references therein)
13	<i>Kluyvera</i>	Janda 2006
14	<i>Legionella</i>	Fliermans et al. 1981
15	<i>Listeria</i>	Sidorenko et al. 2006; Lyautey et al. 2007
16	<i>Morganella</i>	Kitts et al. 1994
17	<i>Pasteurella</i>	Bredy and Botzler 1989
18	<i>Proteus</i>	Hendricks 1972
19	<i>Providencia</i>	Kitts et al. 1994
20	<i>Pseudomonas</i>	Madigan et al. 2009
21	<i>Raoultella</i>	Zadoks et al. 2011 (and references therein)
22	<i>Salmonella</i>	Liang et al. 1982; Gibbs et al. 1997
23	<i>Serratia</i>	Madigan et al. 2009
24	<i>Staphylococcus</i>	Liang et al. 1982; Gunn and Colwell 1983; Soge et al. 2009
25	<i>Stenotrophomonas</i>	Bollet et al. 1995
26	<i>Streptococcus</i>	Gledhill and Casida 1969
27	<i>Yersinia</i>	Sidorenko et al. 2006; Ayyadurai et al. 2008

## References

- Agersø Y, Sandvang D. 2005. Class 1 Integrons and Tetracycline Resistance Genes in Alcaligenes, Arthrobacter, and Pseudomonas spp. Isolated from Pigsties and Manured Soil. *Appl Environ Microbiol* 71:7941-7947.
- Al Saif N, Brazier JS. 1996. The distribution of Clostridium difficile in the environment of South Wales. *J Med Microbiol* 45:133-137.
- Ayyadurai S, Houhamdi L, Lepidi H, Nappez C, Raoult D, Drancourt M. 2008. Long-term persistence of virulent Yersinia pestis in soil. *Microbiology* 154:2865-2871.
- Bollet C, Davin-Regli A, De Micco A. 1995. A Simple Method for Selective Isolation of Stenotrophomonas maltophilia from Environmental Samples. *Appl Environ Microbiol* 61:1653-1654.
- Brandl MT, Haxo AF, Bates AH, Mandrell RE. 2004. Comparison of Survival of *Campylobacter jejuni* in the Phyllosphere with That in the Rhizosphere of Spinach and Radish Plants. *Appl Environ Microbiol* 70:1182-1189.
- Bredy JP, Botzler RG. 1989. The effects of six environmental variables on *Pasteurella multocida* populations in water. *J Wildl Dis* 25:232-239.
- Desmarais TR, Solo-Gabriele HM, Palmer CJ. 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl Environ Microbiol* 68:1165-1172.
- Fliermans CB, Cherry WB, Orrison LH, Smith SJ, Tison DL, Pope, DH. 1981. Ecological distribution of *Legionella pneumophila*. *Appl Environ Microbiol* 41:9-16.
- Gibbs RA, Hu CJ, Ho GE, Unkovick I. 1997. Regrowth of fecal coliforms and salmonella in stored biosolids and soil amended with biosolids. *Water Sci Technol* 35:269-275.
- Gledhill WE, Casida LE. 1969. Predominant Catalase-negative Soil Bacteria. I. Streptococcal Population Indigenous to Soil. *Appl Microbiol* 17:208-213.
- Gunn BA, Colwell RR. 1983. Numerical Taxonomy of *Staphylococci* Isolated from the Marine Environment. *Int J Syst Bacteriol* 33:751-759.
- Hendricks CW. 1972. Enteric Bacterial Growth Rates in River Water. *Appl Microbiol* 24:168-174.
- Hernandez A, Mellado RP, Martínez JL. 1998. Metal accumulation and vanadium-induced multidrug resistance by environmental isolates of *Escherichia hermannii* and *Enterobacter cloacae*. *Appl Environ Microbiol* 64:4317-4320

- Inglis GD, McAllister TA, Larney FJ, Topp E. 2010. Prolonged Survival of *Campylobacter* Species in Bovine Manure Compost. *Appl Environ Microbiol* 76:1110-1119.
- Janda JM, Abbott SL. 2006. The Genus *Hafnia*: from Soup to Nuts. *Clin Microbiol Rev* 19:12-18.
- Janda JM. 2006. New Members of the Family Enterobacteriaceae. In M. Dworkin & S. Falkow, eds. *The prokaryotes: a handbook on the biology of bacteria*. New York: Springer. Pp. 5 - 40.
- Kitts CL, Cunningham DP, Unkefer PJ. 1994. Isolation of three hexahydro-1,3,5-trinitro-1,3,5-triazine-degrading species of the family Enterobacteriaceae from nitramine explosive-contaminated soil. *Appl Environ Microbiol* 60:4608-4611.
- Legender P, Legendre L. 1998. *Numerical Ecology*. Amsterdam:Elsevier Science B.V., 1-853.
- Liang LN, Sinclair JL, Mallory LM, Alexander M. 1982. Fate in model ecosystems of microbial species of potential use in genetic engineering. *Appl Environ Microbiol* 44:708-714.
- Lyautey E, Jackson CR, Cayrou J, Rols, JL, Garabétean F. 2005. Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River watershed, Ontario, Canada. *Appl Environ Microbiol* 73:5401-5410.
- Madigan MT, Martinko JM, Dunlap PV, Clark DP. 2009. *Brock Biology of Microorganisms*. San Francisco:Pearson Benjamin Cummings, 1-1061.
- Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209-220.
- Moriarty E, Nourozi F, Robson B, Wood D, Gilpin B. 2008. Evidence for growth on enterococci in municipal oxidation ponds obtained using antibiotic resistance analysis. *Appl Environ Microbiol* 74:7204-7210.
- Mundt JO. 1963. Occurrence of Enterococci on Plants in a Wild Environment. *Appl Microbiol* 11:141-144.
- Mundt JO. 1961. Occurrence of Enterococci: Bud, Blossom, and Soil Studies. *Appl Microbiol* 9:541-544.
- Rattray EA, Prosser JI, Glover LA, Killham K. 1995. Characterization of rhizosphere colonization by luminescent *Enterobacter cloacae* at the population and single-cell levels. *Appl Environ Microbiol* 61:2950-2957.

- Sidorenko ML, Buzoleva LS, Kostenkov NM. 2006. The effect of soil properties on the preservation and reproduction of Listeria and Yersinia. *Eurasian Soil Sci* 39:211-217.
- Suter II GW, Traas TP, Posthuma L. 2002. Issues and practices in the derivation of species sensitivity distributions. In: *Species sensitivity distributions in ecotoxicology* (Posthuma L, Suter II GW, Traas TP, eds). Florida:Lewis Publishers, 437-473.
- Topp E, Welsh M, Tien YC, Dang A, Lazarovits G, Conn K, et al. 2003. Strain-dependent variability in growth and survival of *Escherichia coli* in agricultural soil. *FEMS Microbiol Lett* 44 44:303-8.
- Vandamme P, Bernardet JF, Segers P, Kersters K, Holmes B. 1994. New perspectives in the classification of the flavobacteria: Description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int J Syst Bacteriol* 44:827-831.
- Zadoks RN, Griffiths HM, Munoz MA, Ahlstrom C, Bennett GJ, Thomas E, et al. 2011. Sources of *Klebsiella* and *Raoultella* species on dairy farms: be careful where you walk. *J Dairy Sci* 94:1045-1051.
- Zaleski KJ, Josephson KL, Gerba CP, Pepper IL. 2005. Potential Regrowth and Recolonization of *Salmonellae* and Indicators in Biosolids and Biosolid-Amended Soil. *Appl Environ Microbiol* 71:3701-3708.